#### REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

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1. AGENCY USE ONLY (Leave blan		3. REPORT TYPE AN	PE AND DATES COVERED		
	23.Mar.05			REPORT	
4. TITLE AND SUBTITLE RECOMBINANT PHAGE PROBES FOR SALMONELLA TYPHIMURIUM DETECTION			5. FÜND	ING NUMBERS	
6. AUTHOR(S)			1		
MAJ OLSEN ERIC V					
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) AUBURN UNIVERSITY MAIN CAMPUS				ORMING ORGANIZATION RT NUMBER	
				CI04-1010	
9. SPONSORING/MONITORING AG		ES)		NSORING/MONITORING	
THE DEPARTMENT OF THE AIR FORCE			AGE	NCY REPORT NUMBER	
AFIT/CIA, BLDG 125					
2950 P STREET					
WPAFB OH 45433					
11. SUPPLEMENTARY NOTES			<u>l</u>		
12a. DISTRIBUTION AVAILABILITY	STATEMENT		I 12h DIS	TRIBUTION CODE	
Unlimited distribution	STATEMENT		125. 5.0	7,1150 11014 0052	
In Accordance With AFI 35-205/	AFIT Sup 1				
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13. ABSTRACT (Maximum 200 wor	ds)		<u> </u>		
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14. SUBJECT TERMS				15. NUMBER OF PAGES	
				16. PRICE CODE	
17. SECURITY CLASSIFICATION 17 OF REPORT	8. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIF OF ABSTRACT	ICATION	20. LIMITATION OF ABSTRACT	
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Type of abstract: Oral, Poster (\* = presenting author).

#### RECOMBINANT PHAGE PROBES FOR SALMONELLA TYPHIMURIUM DETECTION

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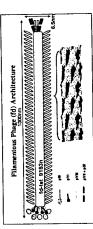
Salmonella typhimurium is a leading cause of inadvertent gastrointestinal foodborne illness in the United States. Although few actual accounts of deliberate food contamination have been documented in the United States, the recent advent of biocrimes and terrorism in our country suggests that this trend will not continue, highlighting the importance of rapidly identifying biological agents, regardless of the contamination origin, as one part of a comprehensive strategic plan to secure the public food supply. There is an urgent need for deployable, real-time threat agent detectors to replace traditional methods of food safety analysis that are slower, labor-intensive, and cost-inefficient. Confirmation of presence in food products can take as long as 48 hours by conventional culture. Current rapid detection initiatives include biosensors that routinely incorporate antibodies as the biorecognition unit. Although sensitive and specific, antibodies are costly and may degrade under unfavorable environmental conditions. We believe that a stable, inexpensive substitute for antibodies is filamentous phage manipulated through phage display technique then affinity selected for specificity to S. typhimurium from billion-clone phage landscape libraries. Our results show that recombinant phage affinity selected against S. typhimurium can be 12,000-22,000 times for more specific than controls and 10-1000 times more selective for S. typhimurium than

other select enterobacteria. We anticipate that these highly specific, selective phage binders will build upon our current biosensor development initiatives for the rapid detection of biological agents such as *S. typhimurium*.

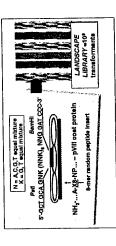
### Introduction

**Binding Confirmation** 

presence in food products can take 48 hours by conventional culture. Current rapid detection initiatives incorporate antibodies as the biorecognition unit. Although sensitive and specific, antibodies are costly and may stable, inexpensive substitute to antibodies is filamentous phage manipulated through phage display technique, then affinity selected for specificity to S. typhimurium from billion-clone phage landscape libraries. Results show that recombinant phage can act as highly specific, sensitive probes for direct typhimurium is a leading cause of foodborne illness in the United States. Confirmation of degrade under unfavorable environmental conditions. biosensors that routinely detection of S. typhimurium. Salmonella a pri l'ou



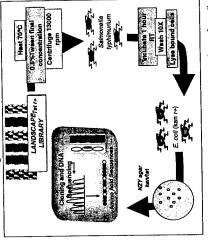
50-residue a-helical subunits of major coat protein pVIII, which overlap one another to form a tube Phage fd-tet outer coat is mainly composed of 4000 encasing the viral single-stranded DNA.



on every subunit of protein VIII using recombinant technique. The foreign peptides are identical in all 4000 subunits of a single virion, but a landscape library as a whole can represent billions of different In a landscape library, foreign peptides are displayed peptides altogether



#### **Precipitation Assay** Phage Selection



binders. Cell bound phage was collected from both eluate and lysate of cells, then amplified in E. coli. DNA of recovered phage clones was used to determine the amino acid sequence of their Following heat incubation (70° C) and depletion of aggregates by precipitation at high centrifugal speed, a phage library was added to a suspension of S. typhimurium for 1 hour to effect cell specific Selection of S. typhimunium specific phage clones from the landscape library was performed by affinity selection in solution. Cells were washed 10X to remove any non-specific respective PVIII displayed peptides.

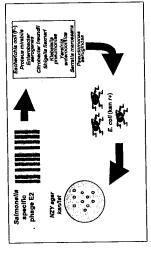
## Phage specificity

("input"). Phage titers were determined by infection of  $E.\ coli$  K91BK strain and described as ratio of output to input phage (% Following recovery of select phage clones, quantitative binding specificity to S. typhimunium was determined for each by precipitation assay ("output") (Results, Fig. 1) in comparison with wild-type vector f8-5, which does not express foreign peptides

Since phage clone E2 demonstrated greatest binding (% Yield) to S. typhimurium, we utilized this phage clone to visually confirm binding specificity. Fluorescent labeling of E2 using Alexa 488 fluorescent tag conferred an estimated 300 molecules of fluorochrome per phage particle. S. typhimunum incubated with the labeled phage was analyzed by fluorescence-activated cell sorting (FACS).

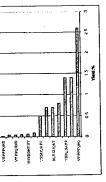
## Phage selectivity

TEM and fluorescence microscopy (data not shown).



typhimurium (Results, Fig. 2). Following incubation of each organism with phage E2, unbound phage were washed from the power of the highest specificity phage clone, E2, for S. typhimurium in comparison to nine other gram-negative bacteria, predominately Enterobacteriaceae selected for their phylogenic relatedness to S. cells, and the cells were then lysed to recover any membrane bound phage. Phage was then transfected into E. coli K91BK for indirect quantitative characterization of direct phage binding to the Precipitation assay was also utilized to confirm the discriminatory challenge bacteria.

## Results



typhimurium were 12,000-22,000 times greater than wild-type control phage f8-5, with phage clone E2 confirmed for S. typhimurium by phage capture, in comparison to ELISA (data not shown), which uses a Fig. 1. Specificity of select phage (10° CFU/ml) were of best clones to efficiency among all select phage tested. the support. Binding

### Conclusions

efforts in the development of phage probes for the landscape phage as substitute antibodies. We anticipate that these highly specific, selective phage development initiatives for the rapid detection of These results confirm our group's previous research build upon our current biosensor biological agents such as S. typhimurium. detection of biological molecules, binders will

# **Acknowledgements**

This work was supported by USDA grant No. 20013439410295C, and ARO/DARPA grant # DAAD 19-01-10454.



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concentration of phage utilized. phage E2 (mean yield Fig. 2. Precipitation assay demonstrated 90% normalized) for S. typhimurium in comparison to challenge bacteria. Mean yield % is an average of 3 separate experiments normalized to a maximal mean yield of 2.8% from S.

typhimunium

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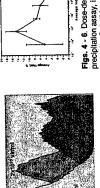


Fig. 3. FACS analysis of phage E2 binding to S. typhimurium. Flurrescence cells treated with phage is greater than that of untreated cells and is dependent upon the

